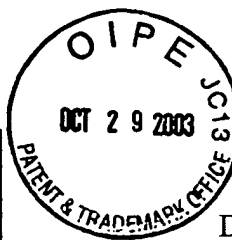


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Dated: 10/29/03

Signature: [Signature]

(Tamara Alcaraz)



Docket No.: 300622007800
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Robert L. ARSLANIAN et al.

Application No.: 09/957,483

Art Unit: 1652

Filed: September 19, 2001

Examiner: K. Kerr

For: PRODUCTION OF POLYKETIDES

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**RESPONSE TO RESTRICTION REQUIREMENT AND
AMENDMENT**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

This is in response to the Office Action dated September 9, 2003 (Paper No. 12), which set forth a restriction requirement for pending claims 1-47 and for which a response was due on October 9, 2003. Filed herewith is a Petition and fee for a one-month extension of time, thereby extending the deadline for response to November 9, 2003. Accordingly, this response is timely filed. Consideration and allowance of the pending claims, as amended, in light of the remarks presented herein are respectfully requested.

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PRELIMINARY AMENDMENT

Prior to examination on the merits, Applicants respectfully request entry of this Preliminary Amendment for the above-captioned patent application. Please amend the claims as follows (claim 40 is amended, claim 41 is cancelled, and claims 48-65 are added):

Claim 1 (withdrawn): A recombinant host cell of the suborder *Cystobacterineae* containing a recombinant expression vector that encodes a heterologous polyketide synthase (PKS) gene and produces a polyketide synthesized by a PKS enzyme encoded on said vector.

Claim 2 (withdrawn): The host cell of Claim 1 that is selected from the family *Myxococcaceae*.

Claim 3 (withdrawn): The host cell of Claim 1 that is selected from the family *Cystobacteraceae*.

Claim 4 (withdrawn): The host cell of Claim 2 that is selected from a genus selected from the group consisting of *Angiococcus*, *Myxococcus*, and *Corallococcus*.

Claim 5 (withdrawn): The host cell of Claim 3 that is selected from a genus selected from the group consisting of *Cystobacter*, *Melittangium*, *Stigmatella*, and *Archangium*.

Claim 6 (withdrawn): The host cell of Claim 4 that is selected from the genus *Myxococcus*.

Claim 7 (withdrawn): The host cell of Claim 5 that is selected from the genus *Stigmatella*.

Claim 8 (withdrawn): The host cell of Claim 6 that is selected from the group consisting of *M. stipitatus*, *M. fulvus*, *M. xanthus*, and *M. virescens*.

Claim 9 (withdrawn): The host cell of Claim 7 that is selected from the group consisting of *S. erecta*, and *S. aurantiaca*.

Claim 10 (withdrawn): The host cell of Claim 8 that is *Myxococcus xanthus*.

Claim 11 (withdrawn): A method for producing a polyketide in a host cell of the suborder *Cystobacterineae*, which polyketide is not naturally produced in said host cell, said method comprising culturing a host cell of Claim 1 under conditions such that a PKS gene encoded on the vector is expressed and said polyketide is produced.

Claim 12 (withdrawn): The recombinant host cell of Claim 1 that produces epothilone or an epothilone derivative.

Claim 13 (withdrawn): The host cell of Claim 10 that produces epothilone or an epothilone derivative.

Claim 14 (withdrawn): The host cell of Claim 13 that produces epothilones A, B, C, and D.

Claim 15 (withdrawn): The host cell of Claim 14 that is *Myxococcus xanthus* K111-32.25.

Claim 16 (withdrawn): The host cell of Claim 14 that produces epothilones A and B as major products and epothilones C and D as minor products.

Claim 17 (withdrawn): The host cell of Claim 13 that produces epothilones C and D as major products.

Claim 18 (withdrawn): The host cell of Claim 17 that either does not contain an *epoK* gene or does not express a fully functional *epoK* gene product.

Claim 19 (withdrawn): The host cell of Claim 18 that is *Myxococcus xanthus* K111-40.1.

Claim 20 (withdrawn): The host cell of Claim 18 that is *Myxococcus xanthus* K111-72.4.4.

Claim 21 (withdrawn): The host cell of Claim 13 that contains an epothilone PKS gene in which a coding sequence for a module of said PKS has been altered by mutation, deletion, or replacement.

Claim 22 (withdrawn): The host cell of Claim 21, wherein said module is extender module 6.

Claim 23 (withdrawn): The host cell of Claim 22, wherein said module lacks a functional ketoreductase domain and produces a 9-keto epothilone.

Claim 24 (withdrawn): The host cell of Claim 21, wherein said module is extender module 5.

Claim 25 (withdrawn): The host cell of Claim 24, wherein said module 5 lacks a functional dehydratase domain and produces a 13-hydroxy epothilone.

Claim 26 (withdrawn): The host cell of Claim 21, wherein said module is extender module 4.

Claim 27 (withdrawn): The host cell of Claim 26, wherein said module lacks a functional ketoreductase domain and produces a 13-keto epothilone.

Claim 28 (withdrawn): The host cell of Claim 21, wherein said module is extender module 2, the coding sequence for the ketosynthase domain has been altered by mutation to change an

active site cysteine to another amino acid, and which host cell must be provided a diketide equivalent compound to produce an epothilone or epothilone derivative.

Claim 29 (withdrawn): The host cell of Claim 28 that is *Myxococcus xanthus* strain K90-132.1.1.2.

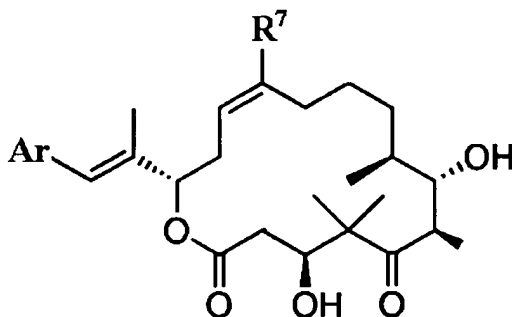
Claim 30 (withdrawn): The host cell of Claim 21, wherein said module is extender module 1.

Claim 31 (withdrawn): The host cell of Claim 30, wherein said module has been changed so that it binds an amino acid other than cysteine.

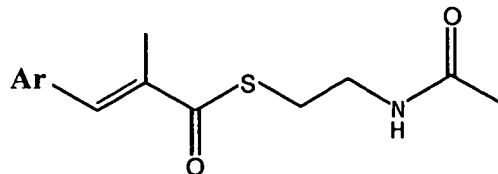
Claim 32 (withdrawn): The host cell of Claim 21, wherein said module is a loading module.

Claim 33 (withdrawn): The host cell of Claim 32, wherein said module has been replaced with a module that binds an amino acid.

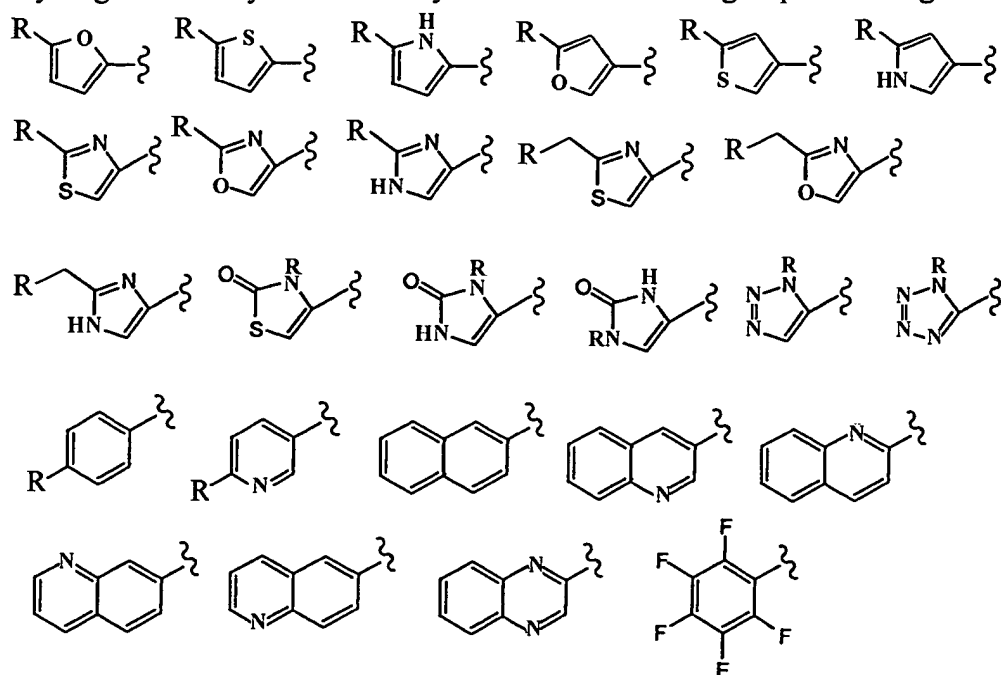
Claim 34 (withdrawn): An epothilone derivative of the formula



produced by culturing said host cell of Claim 28 with a diketide equivalent compound of the formula



where R⁷ is hydrogen or methyl and Ar is aryl is selected from the group consisting of



where R is hydrogen, hydroxy, halogen, amino, C₁-C₅ alkyl, C₁-C₅ hydroxyalkyl, C₁-C₅ alkoxy, and C₁-C₅ aminoalkyl.

Claim 35 (withdrawn): The host cell of Claim 1 that further comprises a heterologous gene that encodes for an enzyme selected from the group consisting of an enzyme that transports a compound into said cell that is utilized in biosynthesis of the polyketide, an enzyme that synthesizes a compound utilized in biosynthesis of the polyketide, and an enzyme that phosphopantetheinylates a PKS.

Claim 36 (withdrawn): The host cell of Claim 35, wherein said enzyme is MatB.

Claim 37 (withdrawn): The host cell of Claim 35, wherein said enzyme is MatC.

Claim 38 (withdrawn): The host cell of Claim 35, wherein said enzyme is MtaA.

Claim 39 (withdrawn): The host cell of Claim 13, wherein said epothilone or epothilone derivative is produced by a PKS gene under the control of a promoter selected from the group consisting of a promoter from an *S. cellulosum* epothilone PKS gene, a promoter from a myxothiazol biosynthesis gene, a promoter from a TA biosynthesis gene, a *pilA* promoter, a promoter from a kanamycin resistance conferring gene, and a So ce90 promoter.

Claim 40 (currently amended): A method for purifying an epothilone from a cell that produces epothilone, said method ~~comprising~~ comprising:

(a) culturing said cell in the presence of ~~XAD~~ a hydrophobic adsorber resin that adsorbs epothilone, under conditions where the cell produces an epothilone; resin;

(b) eluting said epothilone from said ~~resin~~, resin, thereby producing an eluate comprising epothilone; and

(c) performing a solid phase extraction of epothilone from said eluate to produce a composition comprising said epothilone. ~~, and performing chromatography on epothilone resulting from said solid phase extraction.~~

Claim 41 (cancelled)

Claim 42 (withdrawn): Crystalline epothilone D.

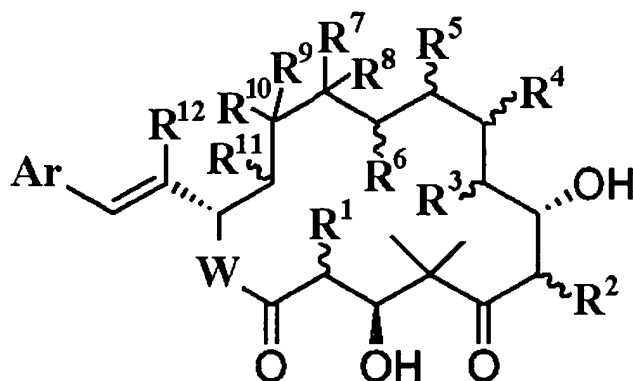
Claim 43 (withdrawn): A method for fermentation of a *Myxococcus* host cell, which method comprises culturing said cell in liquid medium comprising a fatty acid or oil as a carbon source.

Claim 44 (withdrawn): The method of Claim 43, wherein said fermentation is a fed-batch fermentation.

Claim 45 (withdrawn): The method of Claim 43, wherein said *Myxococcus* host cell produces an epothilone or an epothilone derivative.

Claim 46 (withdrawn): The method of Claim 45, wherein said host cell produces an epothilone derivative that contains an oxazole instead of a thiazole, and said liquid medium comprises L-serine.

Claim 47 (withdrawn): A isolated compound of the formula



wherein:

- R^1 , R^2 , R^3 , R^5 , R^{11} , and R^{12} are each independently hydrogen, methyl or ethyl;
- R^4 , R^6 and R^9 are each independently hydrogen, hydroxyl, or oxo; alternatively R^5 and R^6 together form a carbon carbon double bond;
- R^7 is hydrogen, methyl, or ethyl;
- R^8 and R^{10} are both hydrogen or together form a carbon carbon double bond or an epoxide;
- Ar is aryl; and,
- W is O or NR^{13} where R^{13} is hydrogen, C_1 - C_{10} aliphatic, aryl or alkylaryl.

Claim 48 (New): The method of claim 40 further comprising performing chromatography on the composition produced in step (c) of claim 40.

Claim 49 (New): The method of claim 48 wherein the chromatography is performed using a C18 resin with a particle distribution from about 40 microns to about 60 microns.

Claim 50 (New): The method of claim 49 wherein the epothilone is eluted from said resin in an eluent comprising methanol.

Claim 51 (New): The method of claim 48 further comprising a crystallization step.

Claim 52 (New): The method of claim 51 wherein the epothilone is epothilone D.

Claim 53 (New): The method of claim 51 wherein the epothilone is crystallized from a binary solvent system in which water is the forcing solvent.

Claim 54 (New): The method of claim 40 wherein culturing the cell comprises culturing the cell using methyl oleate as a carbon source.

Claim 55 (New): The method of claim 40 wherein the cell is *Sorangium cellulosum*.

Claim 56 (New): The method of claim 40 wherein the cell expresses a heterologous epothilone PKS gene.

Claim 57 (New): The method of claim 56 wherein the cell is from the genus *Myxococcus*.

Claim 58 (New): The method of claim 57 wherein the cell is a *Myxococcus xanthus* cell.

Claim 59 (New): The method of claim 58 wherein the cell is a *Myxococcus xanthus* strain K111-40-1 cell.

Claim 60 (New): The method of claim 40 wherein the hydrophobic adsorber resin is XADTM.

Claim 61 (New): The method of claim 57 wherein the hydrophobic adsorber resin is XAD-16TM.

Claim 62 (New): The method of claim 61 wherein the epothilone is eluted from the hydrophobic adsorber resin by methanol at a concentration of about 100%.

Claim 63 (New): The method of claim 40 wherein the solid phase extraction is preformed using a solid phase that comprises a polystyrene-divinylbenzene resin.

Claim 64 (New): The method of claim 63 wherein the polystyrene-divinylbenzene resin is HP20SSTM.

Claim 65 (New): A method for producing crystalline epothilone comprising:

- (a) culturing a *Myxococcus xanthus* cell in a fed-batch process using methyl oleate as a feed, in the presence of an exogenous trace element solution and a hydrophobic adsorber resin that adsorbs epothilone, under conditions in which the cell produces epothilone;
- (b) eluting the epothilone from said resin using methanol at a concentration of about 100%;
- (c) performing a solid phase extraction of epothilone eluted from said resin using a polystyrene-divinylbenzene resin;
- (d) performing chromatography on the epothilone resulting from the solid phase extraction using a C18 resin with a particle distribution of about 40 microns to about 60 microns; and
- (e) crystallizing the epothilone from a binary solvent system in which water is the forcing solvent.